REMARKS

Specification:

In the Action, the Examiner alleged that page 30 of the specification is missing. Enclosed herewith is a copy of the originally filed page 30 of the specification.

Claim Objections:

Claims 2-8, 10-15 and 22 stand objected because the term "GSK3" was allegedly not defined in the claims. Claims 5,6,14, 15, and 22 stand objected to because the terms "PIP3" and "PI(3,4)P2" were allegedly not defined in the claims. The claims have been amended as suggested by the Examiner to define "GSK3" as "glucose synthase kinase-3", PIP3 as "phosphatidylinositol 3,4,5-triphosphate" and PI(3,4)P2 as "phosphatidylinositol 3,4-biphosphate".

Claim 5 allegedly is a duplicate or substantially a duplicate of claim 22. Applicant would like to defer addressing this issue until claim 5 is found allowable.

35 U.S.C. § 112, second paragraph:

Claims 1-15 and 22 stand rejected under 35 U.S.C. § 112, second paragraph, as being allegedly unclear and indefinite. In particular, the Examiner alleged that part "c" of claim 1 and part "e" of claim 9 do not make clear whether the threonine residue of PKB is phosphorylated. Claims 1 and 9 have been amended to recite "phosphorylated" immediately after "threonine residue" to make clear that both the threonine and the serine are phosphorylated.

The Examiner alleged that claims 2 and 13, which depend from claims 1 and 9, are confusing because they implicitly require a "pre-step" of treating the cells with insulin. Thus, adding insulin in claims 2 and 13 would be redundant.

Applicant respectfully traverse. The specification indicates that the cells need not be grown in the presence of insulin. In such a case, the assay mixture would require the addition of PIP3 or another phosphatidylinositol phosphate compound to the assay mixture. Claim 1 has been amended to optionally include a phosphatidylinositol phosphate compound. Claim 9 already recites in step "d" the presence of the phosphatidylinositol phosphate compound. Support can be found, for example, on page 9, 3rd paragraph, of the specification.

35 U.S.C. § 102(b):

Claims 1-4, 7 and 8 stand rejected for allegedly lacking novelty in light of Wijkander et al. (Journal of Biological Chemistry, 1997, 272(34):21520-21526), Alessi et al. (Current Biology, 1997, 7:261-269) and Cross et al. (Nature, 1995, 378:785-789). The Examiner alleged that Wijkander teaches cytosol fractions and membrane fractions of rat adipocytes, which were combined in a buffer that lacked chloride ions. A PKB assay was performed by addition of ATP and 40 mM MgCl2. Alessi allegedly discloses that full activation of PKB requires phosphorylation of Ser473 and Thr308. Cross allegedly discloses that PKB phosphorylates GSK3.

Applicant respectfully asserts that the claimed invention is novel. First, to anticipate a claimed invention, a single reference must teach each and every element of the claimed invention. The present invention utilizes a reconstituted assay mixture to activate PKB (or Akt). The cells are treated with or without insulin and then fractionated into components: plasma membrane, LDM, and cytoplasm. PKB is not activated unless the membrane fraction and cytoplasmic fraction are brought together and ATP added. Applicant discovered that the membrane fraction contains the kinase necessary to phosphorylate Ser473, and the cytoplasmic

fraction contains the kinase necessary to phosphorylate Thr308. The phosphorylation of both amino acids are required to activate PKB.

In Wijkander, the researchers added insulin to intact rat adipocytes. Then the cells were fractionated into membrane and cytosol components. Each component was tested for PKB activity using a protein kinase assay where radioactive ATP is added. Using the protein kinase assay to determine whether PKB was activated in the intact cell, the authors followed the phosphorylation of two K9 peptides (derived from p70 S6 kinase) with the added radioactive ATP. (p. 21521, 2nd col., 1st para.) The components were not brought back together and the ATP was not utilized to phosphorylate (or activate) PKB itself. Because the membrane and cytosol components were not mixed together to do the assay, the authors were able to conclude (Figure 3) that PKB activity was found mostly in the cytosol. The components were clearly kept and tested separately.

The PKB in the present invention is not activated, even if the intact cells were grown in the presence of insulin (otherwise, it would not be necessary to activate PKB in the assay mixture). Only after the plasma membrane and cytosol components are brought together in the assay mixture and ATP added does PKB get activated (that is, Ser473 and Thr308 are phosphorylated). In contrast, Wijkander showed that PKB is rapidly activated upon stimulation of intact adipocytes with insulin, PKB appeared to be translocated from the cytosol to the membrane to be phosphorylated, and PKB must have returned to the cytosol to perform its function (i.e. phosphorylate its substrate...Figure 3).

Moreover, Applicant has not only reconstituted *in vitro* the dual kinase steps involved in the activation of PKB, but also the insulin signaling pathway leading to the activation of PKB. For example, the present invention successfully reconstituted in vitro the 1) autophosphorylation of the insulin receptor (pp. 1 and 26 of the specification); 2) recruitment of IRS ½ to the plasma membrane and tyrosine phosphorylation of these adapter molecules (pp. 1 and 22); 3) activation of PI 3 kinase after binding to IRS ½ (pp. 23 and 24, Figure 5C); 4) activation of PDK1 as observed by the phosphorylation of threonine on PKB (p. 26); and 5)

activation of PDK2 as observed by the phosphorylation of serine on PKB (p. 29). To the best of Applicant's knowledge, none of these upstream reaction steps leading to the activation of PKB have been successfully reconstituted *in vitro* before the disclosure of the present application.

Neither Alessi nor Cross anticipates the claimed invention. Alessi merely discloses that full activation of PKB requires phosphorylation of Ser473 and Thr308. Cross merely discloses that PKB phosphorylates GSK3. However, merely phosphorylating Ser473, Thr308 and GSK3 do not constitute the claimed invention. Accordingly, none of the cited references teach each and every element of the claimed invention, and thus, cannot anticipate the claimed invention.

35 U.S.C. § 103:

Claims 1-4, 7 and 8 stand rejected as allegedly obvious in light of the combined teachings of Wijkander, Alessi and Cross.

Applicant reasserts its arguments above. Wijkander showed that PKB was already activated in the intact cells. Despite repeated attempts, the researchers could not detect any significant amount of PKB in the membrane. Yet, PKB was supposed to be translocated to the membrane to be phosphorylated or activated. If PKB has already been activated in the intact cells, then translocation to the membrane would not be necessary. The observation is consistent with the authors' assertion that the activation must have occurred rapidly. If so, then by the time the cells were fractionated, the activation would have already occurred, making it unlikely that activation of PKB could be reconstituted *in vitro*. Alessi merely discloses that full activation of PKB requires phosphorylation of Ser473 and Thr308. Cross merely discloses that PKB phosphorylates GSK3. Neither reference cure the deficiency in Wijkander. Therefore, the cited references cannot render obvious the claimed invention.

Claims 1-8 and 22 stand rejected for being allegedly obvious in view of the combined teachings of Wijkander, Alessi, Cross and Vanhaesebroeck (Biochem. J., 2000, 346: 561-576).

Applicant reasserts its arguments above. In addition, Vanhaesebroeck merely shows that the addition of PIP3 and PI(3,4)P2 would enhance the activation of PKB. Dependent claims 5, 6 and 22 recite these two compounds. These claims depend from claim 1, which requires activation of PKB in the assay mixture. Even if Vanhaesebroeck teaches the enhancement of the activation of PKB with PIP3 and PI(3,4)P2, it still does not resolve the deficiency in Wijkander as discussed above. Vanhaesebroeck does not teach the *in vitro* activation of PKB in an assay mixture.

Claims 1-8 and 22 stand rejected for being allegedly obvious in view of the combined teachings of Wijkander, Alessi, Cross and Bauer et al. (General and Comparative Endocrinology, 1983, 49(3):414-427).

Applicant reasserts its arguments above. In addition, Bauer merely shows that the addition of islet cells are insulin-responsive cells and that the claimed method would work equally on islet cells. Dependent claims 7 and 11 recite islet cells. These claims depend from claim 1, which requires activation of PKB in the assay mixture. Bauer does not resolve the deficiency in Wijkander as discussed above. It provides only a general teaching of islet cells and does not teach the *in vitro* activation of PKB in an assay mixture.

Claims 9-15 stand rejected for being allegedly obvious in view of the combined teachings of Wijkander, Hill (Methods Enzymol., 2002, 345: 448-463), Campbell (Biology, 3rd edition, 1992, Benjamin/Cummings Publishing Co., Inc., page 104), Vanhaesebroeck, Alessi and Cross.

Applicant reasserts its arguments above. In addition, Hill merely shows an analysis of subcellular fractions, where the membrane and cytosol fractions may contain PKB. Campell merely shows that the salt concentration (i.e. the chloride concentration) may affect the

enzyme function. Neither reference resolve the deficiency in Wijkander as discussed above. Neither reference teach the *in vitro* activation of PKB in an assay mixture.

Claims 9-15 stand rejected for being allegedly obvious in view of the combined teachings of Wijkander, Hill, Campbell Vanhaesebroeck, Alessi, Cross and Bauer.

Applicant reasserts its arguments above. None of the cited references suggest or teach the claimed invention, an *in vitro* activation of PKB in an assay mixture.

Claims 9-15 stand rejected for being allegedly obvious in view of the combined teachings of Wijkander, Hill (Current Biology., 2002, 12(14): 1251-1255), Campbell, Vanhaesebroeck, Alessi and Cross.

Applicant reasserts its arguments above. In addition, Hill (Current Biology) allegedly show "the use of a high salt solution in order to extract the plasma membrane fraction" and that "the salt-extracted plasma membrane fraction [allegedly] allow for isolation of a constitutively active...PKB...." However, Hill still does not cure the deficiencies in the other cited references, especially Wijkander. Accordingly, none of the cited references suggest or teach the claimed invention, an *in vitro* activation of PKB in an assay mixture.

Claims 9-15 stand rejected for being allegedly obvious in view of the combined teachings of Wijkander, Hill (Current Biology), Campbell Vanhaesebroeck, Alessi, Cross and Bauer.

Applicant reasserts its arguments above. None of the cited references suggest or teach the claimed invention, an *in vitro* activation of PKB in an assay mixture.

CONCLUSION

All of the stated grounds of rejection and objection have been properly traversed, accommodated, or rendered moot. Applicant, therefore, respectfully requests that the Office reconsider all presently outstanding rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action, and as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, she is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Response is respectfully requested.

Respectfully submitted,

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